Changes of Ovarian Structures, Plasma LH, FSH, Progesterone and Estradiol-17β in a Cow with Ovarian Cysts Showing Spontaneous Recovery and Relapse

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ABSTRACT. In a cow diagnosed as having ovarian cysts, we observed changes in the ovarian structures by ultrasonography for 71 days and examined plasma concentrations of sex hormones. The cow had 2 regressing cysts at the start of this study and 3 new follicles subsequently developed into cysts. With regression of these cysts, 2 new follicles developed and ovulated spontaneously, followed by the formation of 2 corpora lutea. On the day prior to ovulation, a preovulatory luteinizing hormone (LH) surge was detected. With regression of the corpora lutea, a new follicle developed and underwent atresia. Meantime, another follicle developed and became a cyst without ovulation. No preovulatory LH surge was observed during the period from regression of the corpora lutea to cyst formation. The results indicate that absence of the preovulatory LH surge is associated with occurrence of ovarian cysts and this endocrine aberration is reversible. — KEY WORDS: cattle, LH surge, ovarian cyst.

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Ovarian cysts in cattle are generally defined as follicular structures of more than 25 mm in diameter which persist for at least 10 days in the absence of a corpus luteum [14]. This ovarian disorder is one of the most important causes of infertility in cattle, and is attended with considerable economic loss because of its high incidence [11]. Cook *et al*. [4] reported that steroid-induced follicular cysts in cattle were not static structures, but regressed to be replaced with other cystic structures. Furthermore, previous investigations [7, 17] showed that spontaneously occurring ovarian cysts also exhibit a turnover of cysts, and suggested that the absence of a preovulatory surge of luteinizing hormone (LH) causes ovarian cysts [3, 7, 17].

Approximately 20% of cows with ovarian cysts recover spontaneously within 30 days following diagnosis [1]. The turnover of cysts and associated changes of plasma gonadotropins and ovarian steroids in cows with spontaneously occurring ovarian cysts have been reported [17], but they have not been reported in cows which recovered from ovarian cysts or then relapsed into cysts. Therefore, the present study is concerned with the changes of follicular and luteal structures and endocrine characteristics with the ovarian cycle in a cow initially diagnosed as having ovarian cysts that ovulated spontaneously and then relapsed into ovarian cysts. In addition, the presence of a preovulatory LH surge was examined during the follicular phase prior to ovulation and recurrence of a cyst.

One Japanese Black cow (10 years old) having ovarian cysts was selected. This cow had calved 10 months prior to this study and had received no treatment for ovarian cysts. The animal was housed in a stanchion stall under natural conditions of sunlight and temperature. It was fed concentrate and hay providing 100% of its requirement for dry matter, 220% of its requirement for digestible crude protein and 100% of its requirement for total digestible nutrients, relative to the maintenance level of the Japanese Feeding Standard for Beef Cattle.

Follicular and luteal structures were examined by transrectal ultrasonography using a 5 MHz transducer and linear-array ultrasound scanner (SSD-280, Aloka, Tokyo, Japan) by the method previously described by Pierson and Ginther [13], and rectal palpation was performed to monitor ovarian changes. At each examination, follicles with a diameter exceeding 10 mm and the location of each structure were recorded on an ovarian map. Ultrasonic examination was made at 3 day intervals. When the cysts or corpora lutea regressed, however, and new follicular structures grew from 10 mm to more than 25 mm in diameter or to ovulation, examination was made daily for 2 series.

Blood samples were collected by jugular venipuncture into heparinized vacutainers at 2 day intervals following the start of ultrasonic examination. An indwelling jugular cannula was used for sampling, when the ovaries were examined daily. All samples were collected between 8:00 and 9:30 hr. The samples were kept on ice and centrifuged at 1,500 g for 30 min at 4°C within 30 min. Plasma was collected and stored at -20°C until the concentrations of LH, follicle-stimulating hormone (FSH), progesterone and estradiol-17β were measured. Additional blood samples were collected via the jugular cannula into plain vacutainers at 3 hr intervals following the start of daily examinations of the ovaries to determine the presence of a preovulatory LH surge. After collection, the samples were kept for 6–18 hr at room temperature. Serum was separated by centrifugation and stored at -20°C until the concentrations of LH were measured.

Plasma and serum concentrations of LH were measured by the homologous double-antibody radioimmunoassay (RIA) method described previously [8] using anti-bovine LH serum (i555/001, UCB-Bioproducts, Braine-l'Alleud, Belgium), bovine LH (i055, UCB-Bioproducts) for radioiodination, and LER-1716–2 (provided by Dr. L. E. Reichert, Albany Medical College) as a reference standard. Plasma concentrations of FSH were measured by RIA [2] using anti-bovine FSH serum (i558/001, UCB-Bioproducts),

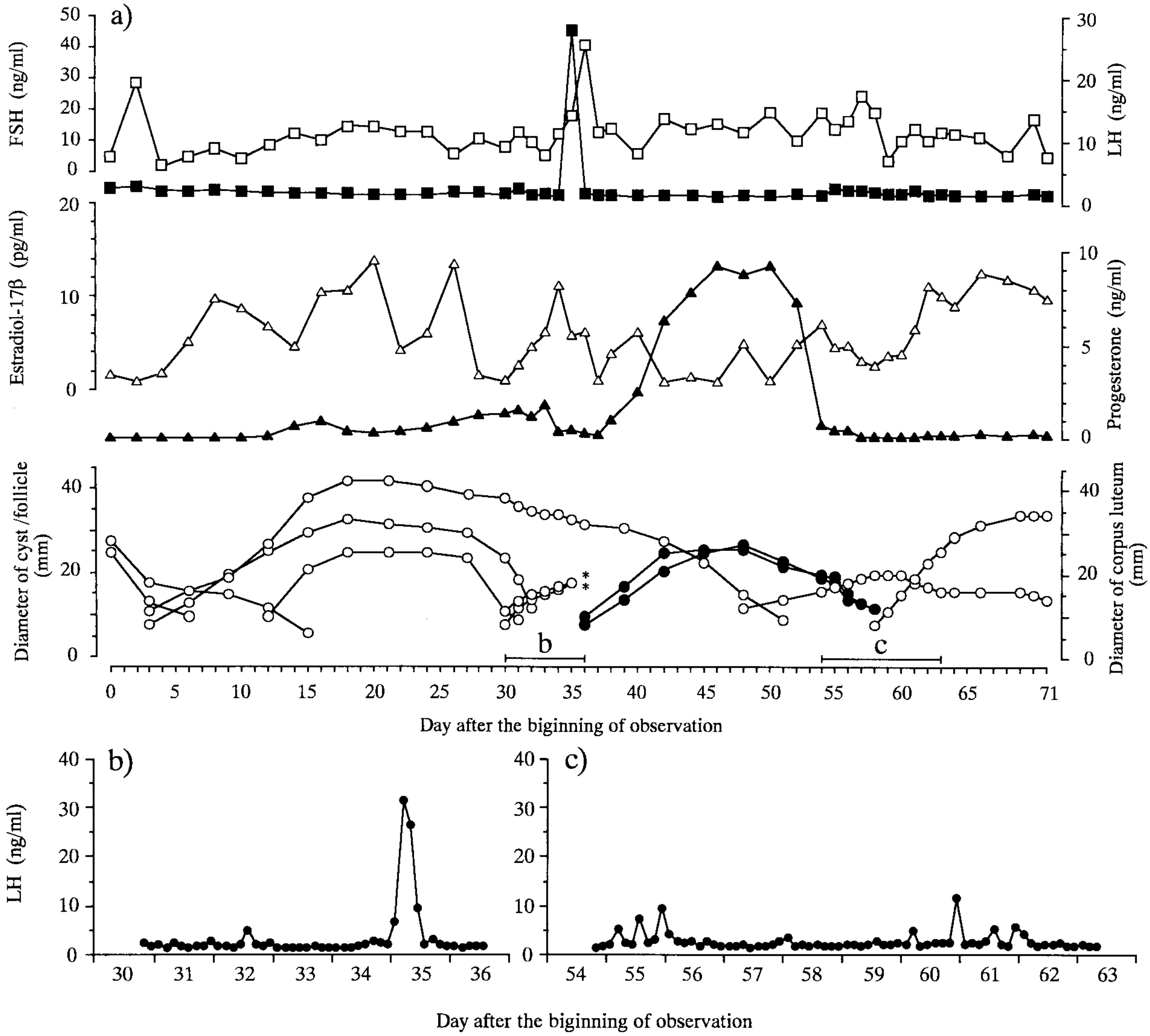

Fig. 1. Plasma concentrations of FSH (□), LH (■), estradiol-17β (△) and progesterone (▲), and diameters of follicular (○) and luteal (●) structures in a cow initially diagnosed as having ovarian cysts (a), with serum concentrations of LH (●) during the developmental stages of follicles followed by ovulation (b), and formation of a cyst (c). This cow exhibited spontaneous ovulation (*) following the regression of cysts and then had a relapse of ovarian cyst. Bars show sampling times; preovulatory LH surge in b) and c) was sought.

USDA-FSH-BP3 for radioiodination, and USDA-FSH-B1 as a reference standard. The sensitivities of the assays for LH and FSH were 0.25 and 2.5 *n*g/m*l*, respectively. The intra- and inter-assay coefficients of variation were 13.0 and 17.2% for LH and 9.9 and 10.5% for FSH, respectively.

Plasma concentrations of estradiol-17β and progesterone were determined by RIA as described by Taya *et al.* [16] using antisera to estradiol-17β (GDN 244) [10] and progesterone (GDN 337) [6]. The sensitivities of the assays for estradiol-17β and progesterone were 0.63 and 12.5 *p*g/m*l*, respectively. The intra- and inter-assay coefficients of variation were 12.8 and 10.6% for estradiol-17β and 5.7 and 9.2% for progesterone, respectively.

The changes in the ovarian structures and hormone concentrations are shown in Fig. 1. This cow had 2 regressing cysts 28 mm and 25 mm in diameter at the start of ultrasonic examination. Then 3 new follicles developed into cysts which also regressed on reaching their maximum diameters, exhibiting a turnover of cysts (Fig. 1a). With regression of these cysts, 2 follicles developed and ovulated spontaneously. Plasma concentrations of progesterone were slightly high (1.1 to 1.8 *n*g/m*l*) from 8 to 3 days before ovulation (Day 28 to 33, Day 0 = the day of the start of ultrasonic examination). Plasma concentration of estradiol-17β increased with the growth of 2 ovulatory follicles, reached a maximum at 11 *p*g/m*l* 2 days before ovulation (Day 34), and then decreased. On the day prior to ovulation (Day 35), the plasma concentration of LH showed a preovulatory LH surge, peaking at 31.3 *n*g/m*l* (Fig. 1b). The ovulated follicles developed into corpora lutea and concurrently there was a marked increase in the progesterone level (≧ 1.0 *n*g/m*l*) from 2 to 16 days after ovulation (Day 37 to 52). With regression of the corpora lutea, a new follicle developed which had a maximum diameter of 20 mm. It did not ovulate and atrophied. Another new follicle appeared and grew into a cyst without ovulation. Due to the regression of corpora lutea, the plasma concentration of progesterone decreased to 0.7 *n*g/m*l* at 18 days after ovulation (Day 54). The plasma concentration of estradiol-17β increased to 7.3 *p*g/m*l* with follicular development, followed by decrease. The level of estradiol-17β then increased with the growth and the cystic formation of a new follicle (about 10 *p*g/m*l*). No preovulatory LH surge was detected during the interval from regression of corpora lutea to cystic formation (Fig. 1c). Plasma FSH levels were about 10 *n*g/m*l* before ovulation, and high (41 *n*g/m*l*) at ovulation. Although FSH levels were slightly high (15 *n*g/m*l*) during the luteal phase, they returned to the 10 *n*g/m*l* level with cystic formation.

In the previous study, we demonstrated that spontaneously occurring ovarian cysts periodically changed their diameter with the stage of the cyst. The ovarian cysts in cattle were characterized by a process of turnover whereby when the original cysts regressed, new follicles developed into cysts on the same or the contralateral ovary [7, 17]. It has been reported that approximately 20% of cows with ovarian cysts recover spontaneously within 30 days following diagnosis [1]. In the present study, a cow initially diagnosed as having ovarian cysts ovulated spontaneously following the regression of cysts and 2 corpora lutea subsequently appeared. There was then relapse into an ovarian cyst following the regression of corpora lutea. In spite of normal levels and patterns of endocrine parameters just before ovulation and during the luteal phase, the reason why cystic ovarian degeneration relapsed is unknown.

An endocrine aberration that could result in the formation of cysts might be the absence or mistiming of the preovulatory LH surge at the developmental stage of follicular structures [9]. Although Cook *et al.* [3] and Hamilton *et al.* [7] reported the absence of a preovulatory LH surge in cows with ovarian cysts, their twice daily sampling regimen was not frequent enough to characterize the intensity or timing of the preovulatory LH surge. In our previous study, we took blood samples at 3 hr intervals during the period when the follicles were growing from 10 to 25 mm in diameter, but no preovulatory LH surge was detected in the cows with ovarian cysts, at least during this developmental stage of the cysts [17]. In the present study, the preovulatory LH surge was observed, when the follicles ovulated. However, it was not detected during the developmental stage of a cyst, when a follicle grew and relapsed into a cyst. These results indicate that lack of the preovulatory LH surge is associated with an appearance of ovarian cysts and this endocrine aberration is reversible.

In normal cows, an elevated circulating concentration of estradiol induces the preovulatory LH surge via positive feedback effects in the hypothalamus and pituitary [15]. Cows with ovarian cysts either lack [5, 12] or have a delayed [18] LH response to estradiol treatment. The positive feedback effects of estradiol-17β thus may not be expressed in cows with cysts. The cow in this study showed the preovulatory LH surge at the time of recovery from ovarian cysts with a high level of estradiol-17β (≧ 10 *p*g/m*l*). However, no preovulatory LH surge could be detected during the developmental stage of a cyst, during which high concentrations of estradiol-17β were also present. Thus, there may have been transient recovery of the positive feedback sensitivity to estradiol in the hypothalamus and pituitary.

Cook *et al.* [3] reported mean serum concentration of LH, pulse frequency and amplitude were higher in cows with cysts than in cows without cysts during the follicular phase. Therefore, occurrence of ovarian cysts may also be associated with the secretory pulse pattern of LH. Further analysis including examination of the secretory pulse pattern of LH should be conducted to clarify the pathogenesis and etiology of cystic ovarian degeneration in cattle.

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REFERENCES

1. Bierschwal, C. J., Garverick, H. A., Martin, C. E., Youngquist, R. S., Cantley, T. C. and Brown, M. D. 1975. *J. Anim. Sci.* 41: 1660–1665.
2. Bolt, D. J. and Rollins, R. 1983. *J. Anim. Sci.* 56: 146–154.
3. Cook, D. L., Parfet, J. R., Smith, C. A., Moss, G. E., Youngquist, R. S. and Garverick, H. A. 1991. *J. Reprod. Fertil.* 91: 19–28.
4. Cook, D. L., Smith, C. A., Parfet, J. R., Youngquist, R. S., Brown, E. M. and Garverick, H. A. 1990. *J. Reprod. Fertil.* 90: 37–46.
5. Dobson, H. and Alam, M. G. S. 1987. *J. Endocrinol.* 113: 167–171.
6. Gibori, G., Anezak, E. and Rothehild, I. 1977. *Endocrinology* 100: 1483–1495.
7. Hamilton, S. A., Garverick, H. A., Keisler, D. H., Xu, Z. Z., Loos, K., Youngquist, R. S. and Salfen, B. E. 1995. *Biol. Reprod.* 53: 890–898.
8. Kamomae, H., Kaneda, Y., Domeki, I. and Nakahara, T. 1988. *Jpn. J. Vet. Sci.* 50: 613–621.
9. Kesler, D. H., Garverick, H. A., Caudle, A. B., Elmore, R. G. Youngquist, R. S. and Bierschwal, C. J. 1980. *J. Dairy Sci.* 63: 166–170.
10. Korenman, S. G., Stevens, R. H., Carpenter, L. A., Robb, M., Niswender, G. D. and Sherman, B. M. 1974. *J. Clin. Endocrinol. Metab.* 38: 718–720.
11. Lopez-Diaz, M. C. and Bosu, W. T. K. 1992. *Theriogenology* 37: 1163–1183.
12. Refsal, K. R., Jarrin-Maldonado, J. H. and Nachreiner, R. F. 1988. *Theriogenology* 30: 679–693.
13. Pierson, R. A. and Ginther, O. J. 1988. *Theriogenology* 29: 21–37.

14. Roberts, S. J. 1986. Veterinary Obstetrics and Genital Diseases, 3rd ed., Edwards Brothers Inc., Ann Arbor.
15. Stumpf, T. T., Wolfe, M. W., Day, M. L., Stotts, J. A., Wolfe P. L., Kittok, R. J. and Kinder, J. E. 1991. *Theriogenology* 36: 201–207.
16. Taya, K., Watanabe, G. and Sasamoto, S. 1985. *Jpn. J. Anim. Reprod.* 31: 186–196 (in Japanese).
17. Yoshioka, K., Iwamura, S. and Kamomae, H. 1996. *Res. Vet. Sci.* 61: 240–244.
18. Zaied, A. A., Garverick, H. A., Kesler, D. J., Bierschwal, C. J., Elmore, R. G. and Youngquist, R. S. 1981. *Theriogenology* 169: 349–358.